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Determination of reaction intermediates and catalytic mechanism by X-ray Diffraction

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Enzyme catalysis has been studied for over a century. How it actually occurs has not been visualized until recently. By combining in crystallo reaction and X-ray diffraction analysis of reaction intermediates, we have obtained unprecedented atomic details of the DNA synthesis and RNA hydrolysis processes. Methods have been developed to co-crystallize native enzyme with cognate substrate but withholding essential metal ions. Enzymatic reaction is triggered by diffusion necessary metal ions into crystals and reaction intermediates are trapped by flash cooling of crystals to below -160°C. Contrary to the established theory that enzyme-substrate complexes and transition states have identical atomic composition and catalysis occurs by the two-metal-ion mechanism, we have discovered that an additional divalent cation has to be captured en route to transition state and product formation. Unlike the canonical two metal ions, which are coordinated by DNA polymerases and RNases, this third metal ion is free of enzyme coordination and is coordinated by nucleic acid substrate/product only. Experimental data indicate that binding of the third metal ion is the rate-limiting step in DNA synthesis and the free energy associated with the metal-ion binding can overcome the activation barrier to the transition state.

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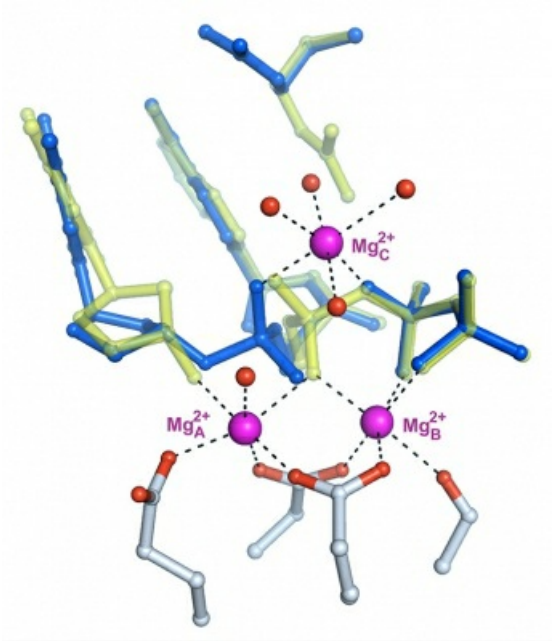

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